Rediscovering nitroxoline: a metal-chelating agent bridging infection and cancer

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Nitroxoline, a legacy antimicrobial agent, is gaining attention for its potential repurposing in infectious diseases and oncology. Its broad-spectrum activity, including biofilm disruption and metal-chelating properties, supports diverse therapeutic applications. However, its systemic use is limited by rapid urinary excretion, short plasma half-life, and limited tissue distribution. In this review, we summarize recent advances in understanding the mechanisms of action, cross-kingdom activity, and anticancer effects of nitroxoline. Despite encouraging preclinical data, clinical translation is constrained by pharmacokinetic (PK) and regulatory challenges. As interest in repurposing established drugs grows, nitroxoline presents a compelling candidate for integration into modern therapeutic strategies across infectious and neoplastic disease domains.

Keywords: nitroxoline; drug repurposing; antimicrobial resistance; metal chelation; biofilm disruption; cancer therapy; pharmacokinetics

Introduction

Global burden of antimicrobial resistance Antimicrobial resistance (AMR) is a major global health threat, jeopardizing decades of medical progress. In 2019, an estimated 4.95 million deaths were associated with AMR, including 1.27 million directly attributable to drug-resistant infections, making it a leading cause of mortality worldwide.(p1) Without intervention, projections suggest the annual global death toll could reach 10 million by 2050.(p2) The burden is particularly severe in low- and middle-income countries (LMICs), where weak healthcare infrastructure and high antibiotic misuse accelerate resistance.(p3) AMR also imposes a substantial economic burden. Recent estimates suggest that, if current trends in drug-resistant infections continue, the global economy could lose more than US$1 trillion annually by 2025.(p4)

Limits of current therapy in an era of resistance

AMR stems from antibiotic overuse and misuse in human and veterinary medicine, inadequate infection prevention, and environmental dissemination of resistance genes. This has led to multidrug-resistant organisms (MDROs), such as carbapenem-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*, rendering many antibiotics ineffective, particularly in intensive care settings.(p5),(p6) For instance, in pediatric and neonatal intensive care units, MDRO bloodstream infections are increasingly common and associated with high mortality, particularly in

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LMICs.[p7] Reports from the Asia-Pacific region, where hospital-acquired infections due to MDROs are especially prevalent, underscore a broader global need for alternative strategies beyond conventional antibiotic development.[p8]

A growing concern is the diminishing efficacy of last-resort antibiotics, such as colistin, now compromised by resistance rates as high as 15% in some clinical settings.[p9] Although colistin is currently used for last-line treatment of multidrug-resistant (MDR) infections, its clinical value is increasingly undermined by both its inherent nephrotoxicity and the global spread of plasmid-borne mobilized colistin resistance (*mcr*) genes, which confer transferable resistance even in intensive care settings.[p10],[p11]

Drug repurposing and global responses to AMR

Recognizing the escalating threat, the WHO has prioritized AMR as a top global public health challenge. Initiatives, such as the Global Action Plan on AMR and the Access, Watch, and Reserve (AWaRe) antibiotic classification system, emphasize the need for coordinated stewardship.[p12],[p13] Regional analyses in resource-limited settings further highlight the need for tailored interventions, such as strengthening microbiology diagnostics, improving local stewardship programs, or prioritizing access to context-appropriate antibiotics.[p14],[p15] In response, innovative strategies, including drug repurposing and targeted antimicrobial delivery, are gaining attention. Repurposing existing drugs offers a cost-effective alternative amid stagnant antibiotic discovery.[p16] One promising candidate is nitroxoline, an 8-hydroxyquinoline compound, which has shown potent activity against MDR *Enterobacteriaceae*.[p17]

Nitroxoline: a rediscovered antibiotic

Historical use and pharmacology

Nitroxoline was developed during the 1950s and has long been used in the treatment of uncomplicated urinary tract infections (UTIs). It received approval in several European countries, particularly Germany, where it remains in clinical use.[p18] Unlike many legacy antibiotics, nitroxoline has retained consistent activity against *Escherichia coli*, the most common uropathogen, with low resistance rates reported over decades.[p19] Its continued efficacy, coupled with a favorable safety profile and oral administration, has supported its use in the treatment of uncomplicated lower UTIs, particularly in women, as well as in the prophylaxis of recurrent infections. Importantly, the established safety profile of nitroxoline in UTI treatment is supported by clinical data showing only mild gastrointestinal disturbances and rare allergic reactions, with no significant difference in adverse event rates compared with controls.[p19]

Renewed interest in nitroxoline has emerged in response to growing resistance to first-line agents, such as trimethoprim-sulfamethoxazole and fluoroquinolones. Contemporary evaluations highlight its value as a safe, cost-effective option in urological practice, despite the relative scarcity of modern clinical trials.[p20] Although its approval is geographically limited, nitroxoline remains a relevant therapeutic agent in regions where it is available and represents a compelling candidate for broader clinical exploration.

Antimicrobial and biofilm-disrupting mechanisms of nitroxoline

Beyond its historical use, nitroxoline exhibits potent activity against a broad spectrum of Gram-negative pathogens, including multidrug-resistant *E. coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, with particular efficacy against carbapenem-resistant *Enterobacterales*.[p21] Its ability to inhibit metallo-β-lactamases (MBLs), such as NDM-1 and VIM-2, through zinc chelation is especially significant, because these enzymes confer resistance to almost all β-lactams. This mechanism, combined with its low resistance selection pressure, makes nitroxoline a promising candidate for repurposing in the context of AMR (Figure 1).

In addition to its activity against planktonic cells, nitroxoline demonstrates a remarkable ability to disrupt bacterial biofilms, complex structures associated with chronic and device-related infections. This applies to pathogens, such as *Pseudomonas aeruginosa*, *E. coli*, and *A. baumannii*.[p22],[p23] This effect is mediated not only by the disruption of biofilm integrity through sequestration of essential metal ions, but also by the induction of bacterial stress responses.[p24],[p25] Together these multifaceted antimicrobial mechanisms support its potential role in the management of recalcitrant infections.

Beyond bacteria: fungal, protozoal, and viral activity

Apart from its established antibacterial profile, nitroxoline demonstrates a striking breadth of activity against fungal, protozoal, and viral pathogens, underscoring its potential as a truly cross-kingdom antimicrobial (Figure 2). In the context of fungal infections, nitroxoline has shown potent *in vitro* activity against *Candida* species, including multidrug-resistant *Candida auris*, with minimum inhibitory concentrations in the lower microgram-per-milliliter range. Its efficacy in azole- and echinocandin-resistant isolates suggests a mechanism distinct from current antifungal classes, potentially involving metal chelation and membrane disruption.[p26]

Notably, nitroxoline has also emerged as a promising candidate for the treatment of free-living amoebae, such as *Acanthamoeba castellanii*, a causative agent of keratitis and granulomatous amoebic encephalitis. Recent transcriptomic and biochemical studies revealed that nitroxoline induces apoptosis in trophozoites and reduces cyst viability, mediated by mitochondrial depolarization, oxidative stress, and interference with iron metabolism and DNA repair pathways.[p27] Similar to its antibacterial effects, the cross-kingdom activity of nitroxoline appears to hinge on conserved mechanisms, such as the above-mentioned metal ion chelation and oxidative stress induction.

In addition, nitroxoline exhibits antiviral activity against mpox virus and severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), inhibiting replication across variants in human epithelial cell lines. The proposed mechanism involves host-directed inhibition of cathepsin B and disruption of endolysosomal trafficking, pointing to a conserved vulnerability across viral pathogens.[p28],[p29] Collectively, these findings expand the therapeutic relevance of nitroxoline far beyond bacterial infections.

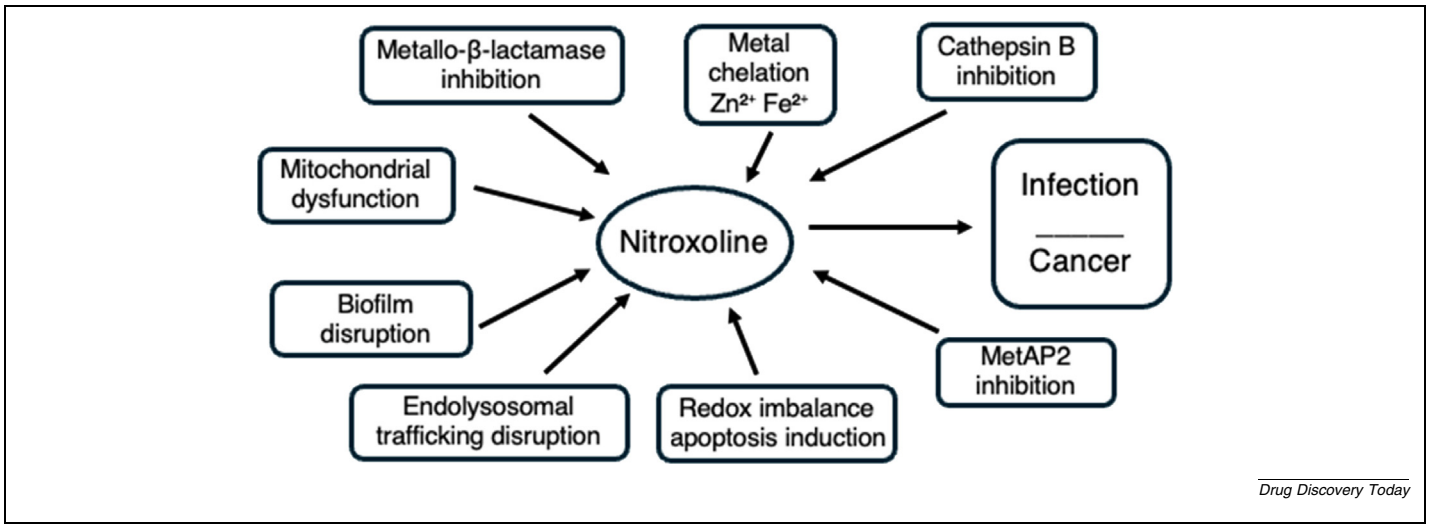

FIGURE 1

Mechanisms of action of nitroxoline across infectious and cancerous contexts. Nitroxoline exerts therapeutic effects through multiple, inter-related mechanisms. These include metal ion chelation (Zn^{2+}, Fe^{2+}), inhibition of metallo-β-lactamases, disruption of microbial biofilms, and induction of redox imbalance and apoptosis. In oncology, nitroxoline also inhibits cathepsin B and methionine aminopeptidase 2 (MetAP2), impairs extracellular matrix integrity, and disrupts mitochondrial function. Its antiviral activity involves inhibition of cathepsin B and interference with endolysosomal trafficking. Together, these diverse mechanisms support the repositioning of nitroxoline as a dual-use agent bridging antimicrobial and anticancer therapy.

Pharmacokinetic barriers to systemic use

Despite this broad pharmacological versatility, the clinical utility of nitroxoline is constrained by PK limitations. The drug is rapidly and nearly completely absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism in the liver, primarily via glucuronidation.[p18],[p20] Consequently, systemic concentrations of the active compound are low, with most of the drug excreted in the urine as conjugated metabolites.[p30] Notably, the generated metabolites appear to retain some antibacterial activity, which contributes to the efficacy of the drug in UTIs.

Nitroxoline also exhibits low plasma protein binding and a limited volume of distribution, resulting in poor tissue penetration.[p31] Consequently, its use remains largely confined to lower UTIs and preclude its effectiveness in treating systemic infections, such as pneumonia or bacteremia (Figure 3). Based on these findings, there is growing interest in strategies to improve the PK profile of nitroxoline and broaden its clinical applications.

Nitroxoline in oncology

Multifaceted mechanisms and translational repurposing

In addition to its antimicrobial activity, nitroxoline has demonstrated consistent anticancer effects across *in vitro* and *in vivo* models, driven by modulation of key oncogenic pathways involved in tumor growth, metastasis, and treatment resistance. One of its most well-characterized actions is inhibition of the transcription factor STAT3, a central regulator of tumor cell survival, immune evasion, and chemoresistance. In drug-resistant urothelial bladder cancer models, nitroxoline downregulated phosphorylated STAT3 and its downstream effectors, including c-Myc, Bcl-xL, Cyclin D1, and Mcl-1, leading to G0/G1 cell cycle arrest and apoptosis in both *in vitro* and *in vivo* systems.[p32]

In parallel, nitroxoline has been shown to inhibit BRD4, a bromodomain-containing protein involved in MYC-driven transcriptional programs. This activity enhanced bortezomib sensitivity and induced mitochondrial-mediated apoptosis in multiple myeloma cells.[p33] Nitroxoline also inhibits SIRT1 activity, a NAD^+-dependent deacetylase, in endothelial cells, a mechanism associated with impaired angiogenesis and reduced tumor growth in xenograft models of bladder and breast cancer.[p34] Cathepsin B inhibition by nitroxoline further contributes to its anticancer effects, curbing extracellular matrix degradation, cell migration, and invasion in models of breast and bladder cancer.[p35],[p36] To improve this activity, derivatives with modifications at positions 5 and 7 of the quinoline ring were synthesized, showing nanomolar cathepsin B exopeptidase inhibition and reduced tumor cell invasiveness *in vitro*.[p37] Further evidence demonstrates that nitroxoline analogs targeting cathepsin B suppress extracellular matrix degradation and tumor cell invasion in functional cancer models.[p38] In addition to cathepsin B, nitroxoline has also been shown to inhibit methionine aminopeptidase 2 (MetAP2), a metalloprotease involved in angiogenesis and cell proliferation. This interaction has been associated with anticancer effects *in vitro* and in preclinical models.[p39]

Subsequent mechanistic studies reveal that the anticancer effects of nitroxoline in pancreatic and renal carcinoma models are linked to redox imbalance, mitochondrial dysfunction, and iron homeostasis disruption, mechanisms that may raise concerns about potential toxicity, yet preclinical data thus far indicate selective action against tumor cells with tolerable *in vivo* profiles.[p40],[p41] Notably, the doses used in preclinical cancer models are significantly higher than those used for UTIs; however, so far, these elevated doses have demonstrated favorable tolerability *in vivo*, suggesting a potential therapeutic window. Together, these findings underscore the mechanistic breadth of

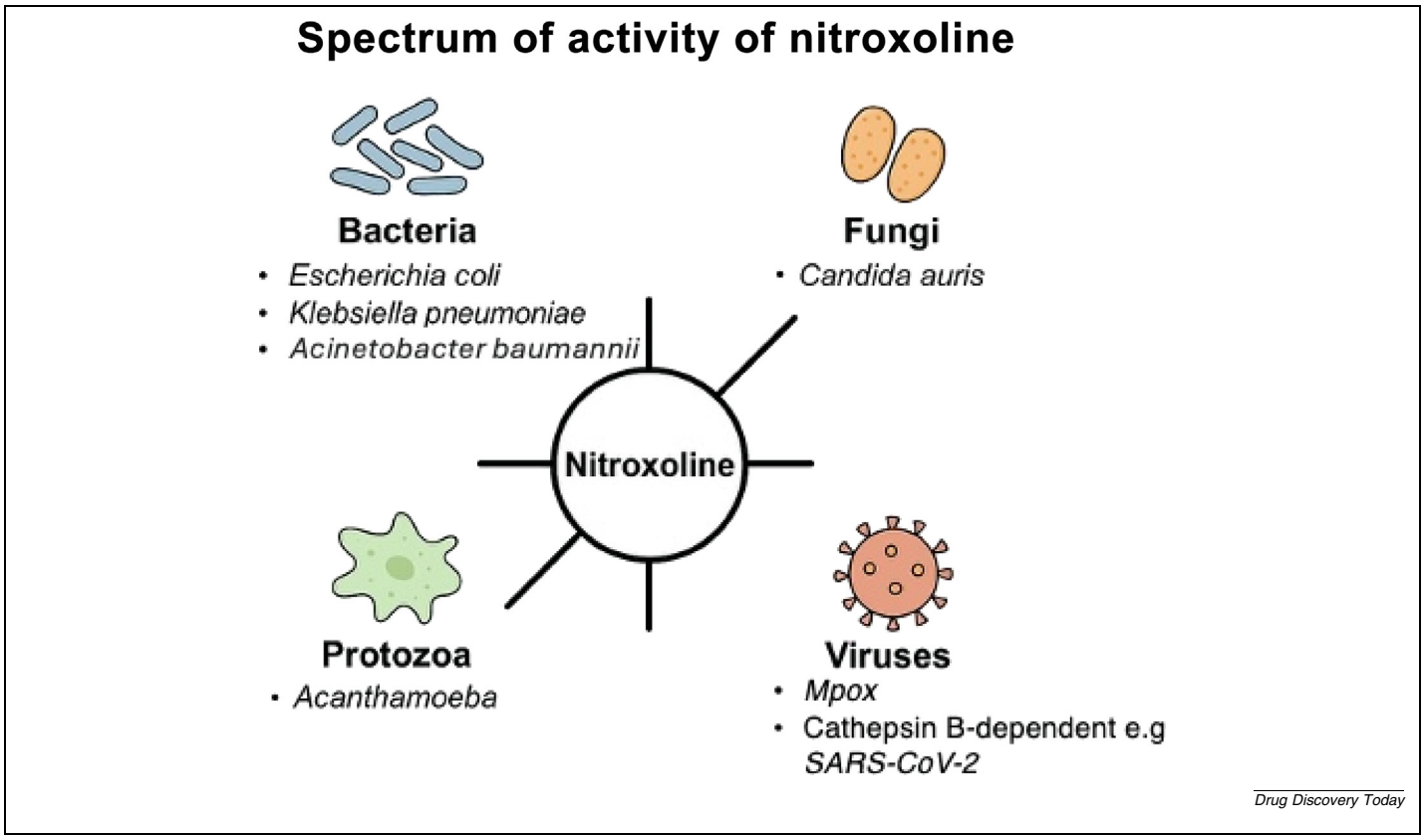

FIGURE 2

Broad-spectrum antimicrobial activity of nitroxoline. Nitroxoline demonstrates potent cross-kingdom activity against diverse pathogens. It is active against bacteria (e.g., *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*), fungi (e.g., *Candida auris*), protozoa (e.g., *Acanthamoeba*), and viruses [e.g., as mpox and severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2)]. Its antiviral activity is partly mediated by cathepsin B inhibition and disruption of endolysosomal trafficking. This broad spectrum underscores the therapeutic versatility of nitroxoline and the potential to repurpose it in infectious disease management.

nitroxoline and support its potential as a lead compound for the development of multitargeted cancer therapies, although its clinical translation will require careful optimization to address PK limitations and ensure selective safety.

Evidence from preclinical cancer models

Preclinical evaluations of nitroxoline demonstrated anticancer activity across a range of tumor types. For instance, in bladder cancer, nitroxoline inhibited tumor progression in orthotopic and subcutaneous xenograft models by suppressing epithelial–mesenchymal transition (EMT), reducing tumor-associated myeloid-derived suppressor cells (MDSCs), and enhancing antitumor immunity.[p36],[p42] These effects translated into significantly reduced tumor volumes and metastasis rates.

Promising anti-cancer activity has also been observed in pancreatic ductal adenocarcinoma, where nitroxoline and its derivatives exhibited cytotoxicity across multiple cell lines, with structural optimization yielding improved potency and selectivity.[p43] In an extended screening effort, a large series of nitroxoline analogs were evaluated, revealing multiple derivatives with enhanced antiproliferative activity and defined structure–activity relationships.[p43] Similarly, glioblastoma models showed tumor growth inhibition and increased apoptosis following nitroxoline treatment, as demonstrated by MRI-based tumor volume measurements and TUNEL analysis in a PTEN/KRAS mouse model.[p44]

In prostate cancer, combination therapy with anti-PD-1 immunotherapy and nitroxoline resulted in tumor regression and increased CD8+ T cell infiltration, indicating potential for synergistic immunomodulation.[p45] Additional studies in breast and renal cancers support its broad-spectrum applicability, with liposomal formulations improving both bioavailability and tumor accumulation.[p35],[p41] Preclinical studies indicate that the anticancer efficacy of nitroxoline may be achieved without substantial systemic toxicity. For instance, in mouse models of bladder and renal cancer, treatment with nitroxoline significantly reduced tumor growth without causing weight loss or increased mortality, even at higher doses.[p31] This supports its potential safety profile in oncological applications and aligns with the favorable tolerability observed in clinical use for infectious diseases. Collectively, these preclinical findings reinforce the mechanistic diversity of the anticancer effects of nitroxoline and highlight its potential for repurposing across distinct oncological indications (Table 1).

TABLE 1

Summary of anticancer activity of nitroxoline in preclinical models

Cancer type	Mechanism(s) of action	Model system	Delivery strategy	Key outcomes	Refs
Bladder	STAT3 inhibition; EMT reversal; Cathepsin B inhibition; immune modulation	*In vitro*, xenograft, orthotopic mouse models	Free drug	Tumor growth suppression; reduced metastasis; enhanced antitumor immunity	(p32), (p36)
Breast	Cathepsin B inhibition; anti-migration	*In vitro*, xenograft mouse models	HER2-targeted liposomes (co-delivered with cisplatin)	Increased cytotoxicity; improved tumor inhibition; reduced systemic toxicity	(p35)
Endothelial driven (e.g., breast, bladder)	MetAP2 inhibition by nitroxoline analogs	*In vitro,* xenograft mouse models	Free drug	Reduced angiogenesis; suppressed tumor growth and proliferation	(p39)
Glioblastoma	APE1 downregulation; mitochondrial disruption	*In vitro*, *in vivo* mouse models	Free drug	Induced apoptosis; reduced tumor volume; increased therapeutic sensitivity	(p42), (p52)
Lung	MDM2 degradation	*In vitro*	Free drug	Induced apoptosis	(p53)
Multiple myeloma	BRD4 inhibition; mitochondrial apoptosis	*In vitro*, xenograft mouse models	Free drug	Enhanced sensitivity to bortezomib; apoptosis induction	(p33)
Pancreatic	Redox disruption; mitochondrial dysfunction	*In vitro* (AsPC-1), various cell lines	Structural derivatives (e.g., ASN-1733)	Increased potency and selectivity; growth inhibition; redox imbalance	(p40), (p43)
Prostate	Immunomodulation; synergy with PD-1 blockade	Mouse models	Combination therapy with PD-1 inhibitor	Tumor regression; enhanced CD8+ T cell infiltration	(p45)
Renal cell	BET inhibition; mitochondrial stress	*In vitro*, xenograft mouse models	Nitroxoline-derived BET inhibitors	Tumor growth suppression; improved formulation bioavailability	(p41)

Targeting metal-dependent pathways in infection and cancer

Advances in molecular pharmacology have revealed that the diverse therapeutic effects of nitroxoline are underpinned by a common set of biochemical mechanisms relevant to both infection and cancer biology.(p46)

As mentioned above, the unifying feature of the biological activity of nitroxoline is its ability to chelate divalent metal ions, such as Zn^{2+} and Fe^{2+}, a property central to both its antimicrobial and anticancer effects. In bacteria, metal chelation inhibits metallo-β-lactamases, restoring β-lactam susceptibility in resistant strains.(p47) The same principle underlies its biofilm-disrupting effects in *P. aeruginosa*, where metal deprivation triggers structural collapse.(p48) In experimental cancer models, the interference of nitroxoline with intracellular zinc and iron homeostasis perturbs metal-dependent enzymatic activity at the protein level, including cathepsin B and histone deacetylases, contributing to apoptosis and metabolic stress.(p49) This cross-domain mechanism underscores the therapeutic versatility of nitroxoline.(p50)

Building on its metal-chelating activity, nitroxoline also interferes with redox homeostasis and mitochondrial function, which are central stress-response pathways in both pathogens and tumor cells. In pancreatic cancer cells, it induces mitochondrial depolarization, generates reactive oxygen species (ROS), and activates the DNA damage response, contributing to apoptosis and metabolic arrest.(p40) In *A. castellanii*, nitroxoline induces an oxidative imbalance and alters redox-sensitive gene expression, including downregulation of iron metabolism and mitochondrial pathways.(p27) Related effects have been observed in multiple myeloma, where nitroxoline triggers ROS accumulation and mitochondrial-mediated cell death.(p33) Furthermore, in neuroprotective models, nitroxoline was shown to preserve mitochondrial integrity and limit oxidative stress under ischemic conditions.(p51) These findings highlight redox and mitochondrial disruption as conserved targets of nitroxoline across therapeutic domains.

In addition to disrupting redox balance and mitochondrial function, nitroxoline initiates conserved stress signaling cascades that culminate in programmed cell death across microbial and cancer models. In glioblastoma and lung cancer cells, it induces apoptosis through mitochondrial membrane disruption, ROS accumulation, and degradation of anti-apoptotic regulators, such as APE1 and MDM2.(p52),(p53) Similarly, protozoal models of *A. castellanii* reveal hallmark features of regulated cell death, including chromatin condensation, mitochondrial dysfunction, and DNA fragmentation, following nitroxoline exposure.(p54) These effects position apoptosis and stress signaling as downstream convergence points of the multi-targeted activity of nitroxoline.

Taken together, these findings support nitroxoline as a rare example of a small molecule that exerts multi-targeted activity across therapeutic domains. Rather than indicating a true mechanistic convergence between infection and cancer, its broad-spectrum efficacy appears to stem from disruption of metal ion homeostasis, a vulnerability shared by many pathogenic and malignant cells. These properties position nitroxoline as a versatile repurposing candidate across infection and oncology. While preclinical data are compelling, the therapeutic value of nitroxoline in oncology remains largely untapped in the clinic. Future studies will need to clarify optimal dosing, delivery strategies, and combination regimens, while also advancing structure–activity relationship studies to optimize target selectivity and minimize off-target effects in oncology. As the boundaries between antimicrobial and anticancer mechanisms continue to blur, nitroxoline may emerge as a versatile agent in both fields.

However, realizing this potential in clinical practice will depend on overcoming regulatory, PK, and perceptual barriers that currently limit its broader medical application.

Mechanisms of resistance to nitroxoline

Intrinsic resistance and susceptibility patterns

Although the clinical use of nitroxoline has historically focused on UTIs, characterizing the full extent of its antimicrobial spectrum remains key to assessing opportunities for therapeutic repurposing. As detailed above, its unique chelation-driven mechanism targets a range of pathogens, but activity levels can vary significantly between species as a result of intrinsic resistance mechanisms.

Recent large-scale susceptibility profiling demonstrated that nitroxoline exhibits potent activity against numerous Gram-negative pathogens, including members of the *Enterobacterales* and *Moraxellaceae*. Many of these organisms show inhibition at concentrations considered well within the therapeutically achievable range.[p55] In particular, clinical isolates of *A. baumannii*, *Salmonella enterica*, and *K. pneumoniae* often respond to nitroxoline at levels suggesting these species are intrinsically susceptible.

However, its activity is not universal. Species such as *P. aeruginosa* often show low-level responsiveness, with many strains requiring concentrations beyond clinically achievable thresholds.[p56] Other species, including *Serratia marcescens*, *Proteus vulgaris*, and *Enterobacter cloacae*, exhibit variable susceptibility profiles.

Among Gram-positive organisms, nitroxoline demonstrates moderate activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and coagulase-negative staphylococci. However, there is greater heterogeneity in responsiveness among these groups. Interestingly, it has also been shown to inhibit less common uropathogens, such as *Aerococcus urinae* and *Aerococcus sanguinicola*, indicating potential utility beyond the classical enteric and staphylococcal pathogens.[p57]

Diagnostic gaps and breakpoint limitations

Despite this broad intrinsic activity, current clinical microbiology practices do not fully accommodate nitroxoline. Presently, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) provides a clinical breakpoint only for *E. coli* in the treatment of uncomplicated UTIs. Breakpoints for other bacterial genera, such as nonfermenters and Gram-positive cocci, remain undefined. In parallel, Clinical & Laboratory Standards Institute (CLSI) panels do not include nitroxoline, and disc diffusion guidance is limited. These gaps hinder standardized susceptibility testing, complicating its integration into routine antimicrobial stewardship programs. Together, these observations support a generally favorable intrinsic activity profile for nitroxoline, with notable exceptions. However, the absence of standardized interpretive criteria across species underscores the need for more robust surveillance and harmonization. Furthermore, this diagnostic uncertainty highlights the requirement for clearer interpretive criteria, especially as we consider acquired resistance, the subject of the next section.

Acquired resistance mechanisms

Resistance to nitroxoline has proven difficult to select and sustain, in both clinical surveillance and laboratory evolution models. Long-term adaptation experiments in *E. coli* and *K. pneumoniae* revealed that resistance requires sequential mutations in global regulators rather than mutations in a discrete drug-binding site. Most commonly, resistance arises from loss-of-function mutations in *emrR*, the repressor of the EmrAB-TolC efflux system, with further adaptation involving *marR*, *lon*, or *envZ*, genes involved in membrane regulation, stress response, and porin modulation.[p58],[p59] These findings point to a polygenic basis of resistance, likely reflecting the multitarget impact of the compound on bacterial homeostasis, including disruption of metal regulation and membrane integrity.[p55]

Even under optimized laboratory conditions, the frequency of spontaneous resistance mutations remains extraordinarily low. No detectable nitroxoline-resistant mutants were recovered in standardized selection assays, whereas comparators, such as trimethoprim or pivmecillinam, readily produced resistant colonies under the same conditions.[p58] Similarly, in systematic assays with hundreds of strains, resistance to nitroxoline remained exceptionally uncommon.[p55],[p59] This mirrors clinical data: a surveillance study found 100% of uropathogenic *E. coli* isolates to be susceptible to nitroxoline, even amid ongoing outpatient use.[p60]

Resistance costs and stewardship implications

In rare cases where resistance emerged, its stability proved fragile. Mutants displayed significant physiological costs, including slower growth, impaired motility, and severely reduced virulence, as shown, for instance, in a zebrafish infection model.[p58] These effects are likely a consequence of broad metabolic disruptions linked to efflux overexpression and membrane remodeling. Importantly, many resistant strains showed signs of reversion under drug-free conditions, reinforcing the notion that resistance to nitroxoline is not only rare, but also evolutionarily unstable.

Clinically, this resistance profile underscores the strategic value of nitroxoline in stewardship-focused treatment models. The high barrier to resistance supports the repositioning of nitroxoline in treatment algorithms for uncomplicated UTIs, especially those involving MDR pathogens. At the same time, its over-the-counter availability in some markets raises concerns about unsupervised use. Broader diagnostic support, harmonized susceptibility testing, and rational prescribing will be essential to maintain its efficacy.

Taken together, the data suggest that the multifaceted mode of action of nitroxoline and the fitness burden of resistance limit its vulnerability to adaptation, strengthening the case for reintegration into clinical care, provided that systemic barriers are addressed through policy, surveillance, and stewardship.

Barriers to broader clinical use

Regulatory and market access barriers

Despite promising preclinical data and decades of safe clinical use in certain countries, nitroxoline remains underutilized due

to a combination of regulatory inertia, commercial disinterest, and unresolved PK limitations. Its current approvals are geographically restricted and largely confined to UTIs. Broader registration efforts have stalled, in part, because nitroxoline is off-patent, limiting financial incentives for pharmaceutical investment or reformulation. Nonetheless, its unique mechanism of action and low resistance profile make nitroxoline a promising lead compound for further development, particularly through structural modification or pharmaceutical reformulation aimed at improving PK and expanding its therapeutic potential beyond localized infection [(p58)]

As a result, repurposed drugs including nitroxoline often fall into a regulatory gray zone. Although off-label use is technically possible, formal approval for new indications requires costly, time-intensive clinical trials, which are rarely pursued without the prospect of exclusivity or market return.[(p61)] However, locally confined diseases, such as bladder cancer, can represent viable opportunities for clinical repurposing without structural modification, particularly where intravesical delivery can circumvent PK limitations. In this context, nonprofit and academic initiatives have a crucial role, although they often lack the infrastructure for large-scale regulatory engagement. Mechanisms, such as the EU's Pediatric Use Marketing Authorisation (PUMA), orphan drug designation, and scientific advice programs, have successfully facilitated repurposing in other therapeutic areas. However, such pathways remain underleveraged in infectious disease repurposing, especially for generically available agents such as nitroxoline.[(p62)]

Geographical disparities in approval and access

The development of nitroxoline for broader infectious or oncological applications will likely depend on coordinated public–private partnerships, regulatory flexibility, and policy-level recognition of the unique barriers facing off-patent repurposing candidates. Although nitroxoline has been in clinical use for decades, its approval status varies widely across countries. Currently, the drug is approved in only a handful of European countries, including Germany, Romania, Bulgaria, and Poland, where it is primarily indicated for the treatment of uncomplicated UTIs. Marketing authorization has been withdrawn in others, such as France, despite no major safety concerns.[(p58)] No formulation of nitroxoline has ever received regulatory approval in the USA.

In the absence of US Food and Drug Administration (FDA) approval, nitroxoline is accessible in the USA only through an Expanded Access Investigational New Drug (IND) program, managed by the Centers for Disease Control and Prevention (CDC). This pathway permits emergency use of the drug in life-threatening infections caused by free-living amoebae, such as *Balamuthia mandrillaris* and *Naegleria fowleri*. Although promising case reports exist, this restricted-access framework highlights the broader regulatory constraints on its clinical use.

Furthermore, susceptibility testing guidance also varies significantly across regions. Whereas the EUCAST has issued rationale documents and a breakpoint for nitroxoline, these are not universally adopted and remain absent from major global guidelines.[(p56)] Thus, the lack of harmonized regulatory and clinical frameworks continues to delay broader integration of nitroxoline into routine practice worldwide.

Global guideline gaps for repurposed agents

Legacy antibiotics, such as nitroxoline, often fall outside the scope of major international guidelines, not because of efficacy concerns, but due to entrenched regulatory barriers. Despite its favorable resistance profile and long-standing clinical use in parts of Europe, nitroxoline is not listed in the WHO's Essential Medicines List (EML) or included in the AWaRe classification, which shapes global prescribing and procurement practices.[(p46)]

As noted, the FDA has not approved nitroxoline for any indication, which precludes its inclusion in US clinical guidelines.[(p63)] By contrast, the European Medicines Agency (EMA) has acknowledged the broader challenges of re-evaluating legacy agents, and launched initiatives, such as the Safe and Timely Access to Medicines for Patients (STAMP) working group, to support repurposing efforts. However, regulatory structures for off-patent drugs remain poorly coordinated, with limited incentives for applicants to pursue broader authorization.[(p64)]

Without harmonized international recognition or integration into essential medicine frameworks, nitroxoline remains an underleveraged therapeutic option. Modernizing regulatory frameworks to support legacy drug repurposing is key to unlocking their full clinical potential.

Barriers to clinical trial advancement

Despite promising interim results from a randomized Phase II trial evaluating nitroxoline (APL-1202) in combination with the PD-1 inhibitor tislelizumab for muscle-invasive bladder cancer (MIBC), the clinical development of nitroxoline remains in an early phase, with the trial limited by small sample size, retrospective eligibility issues, and a substantial number of patients declining surgery.[(p65)] Although several preclinical studies have demonstrated promising anti-tumor effects across various cancer models, including glioma, lymphoma, and pancreatic cancer, the translation of these findings into robust clinical programs has been minimal.[(p31)]

This gap between preclinical promise and clinical validation reflects broader challenges in drug repurposing for oncology. The longstanding use of nitroxoline in infectious disease supports its safety profile, but this has not translated into widespread adoption in cancer trials, largely due to unresolved PK challenges. Without structural optimization or improved formulation, its *in vitro* efficacy is unlikely to yield durable clinical benefits, especially for systemic indications. Regulatory and financial barriers further limit progress, particularly for off-patent agents with low commercial incentives for investment in large, multicenter trials. Furthermore, most existing studies use single-arm designs or are limited to early-phase cohorts, hindering the generation of high-quality, comparative evidence needed for regulatory approval and clinical uptake.[(p36)]

As a result, nitroxoline remains a compelling yet underdeveloped candidate in oncology. Overcoming these clinical trial limitations will require concerted efforts to build collaborative research frameworks and funding mechanisms that can support rigorous evaluation in cancer-specific settings.

Overcoming pharmacokinetic barriers: optimization and formulation strategies

One reason why the clinical repurposing of nitroxoline beyond the urinary tract has been limited is its unfavorable PK profile. As mentioned above, orally administered nitroxoline is rapidly absorbed and primarily excreted in urine, mostly as conjugates, resulting in a short plasma half-life of ~1.8 h and limited systemic exposure.[p30] This PK profile limits its utility against systemic malignancies, where effective tissue penetration and sustained exposure are essential.

To overcome these pharmacological hurdles, several strategies have been explored. Nanoparticle-based formulations, in particular, have shown promise in preclinical oncology models. To this end, HER2-targeted liposomes coloaded with nitroxoline and cisplatin have been developed for use in HER2-positive breast cancer, increasing cellular uptake and cytotoxicity while suppressing tumor cell migration. *In vitro*, these formulations showed greater apoptosis induction and lower IC_{50} values compared with nontargeted or free drug combinations. *In vivo*, this approach let to superior tumor growth inhibition without added systemic toxicity, underscoring the value of HER2-specific targeting and liposomal delivery for enhancing the anticancer efficacy of nitroxoline.[p35]

Efforts to improve the physicochemical and pharmacokinetic profiles of nitroxoline have included both solvent system modeling and chemical derivatization. Whereas solubility optimization using mixed organic solvents has offered foundational insights into delivery feasibility, more significant translational advances have emerged through structural modification of the nitroxoline scaffold. Among these, ASN-1733 demonstrated markedly enhanced systemic exposure, prolonged plasma half-life, increased maximum concentration (Cmax), and improved bioavailability compared with the parent compound.[p66] It maintained broad-spectrum activity against multidrug-resistant *Enterobacterales* and exhibited robust therapeutic efficacy in multiple murine infection models, including sepsis and abdominal infections, indications where unmodified nitroxoline is ineffective. Mechanistic studies revealed that ASN-1733 operates via dual functionality: disrupting bacterial outer membranes through calcium ion chelation and inhibiting NDM-1 by binding to Loop10 residues, thereby blocking substrate access to the active site of the enzyme even under high-zinc conditions. Although not yet applied in cancer models, these findings underscore the feasibility of rationally designing nitroxoline analogs with improved absorption, prolonged circulation, and enhanced mechanistic potency for systemic applications.[p67]

Further advances in nitroxoline delivery will likely depend on a combination of drug reformulation and targeted delivery technologies. Given its well-established safety profile and anticancer mechanisms, such as cathepsin B inhibition and anti-angiogenic activity, these innovations could enable broader clinical applications for nitroxoline in oncology settings.

Collectively, these barriers, regulatory, commercial, and infrastructural, continue to hinder the broader clinical adoption of nitroxoline, despite strong preclinical and historical safety data. Addressing them will require coordinated action across regulatory agencies, public–private partnerships, and international health organizations. Without such reforms, the full therapeutic potential nitroxoline could remain unrealized in both infectious disease and oncology.

Concluding remarks

Nitroxoline exemplifies the untapped potential of legacy compounds in addressing some of today's most urgent medical challenges. With demonstrated activity against a diverse array of bacterial, fungal, protozoal, and viral pathogens, as well as a growing body of evidence supporting its anticancer effects, it stands out as a rare cross-domain therapeutic candidate. Its unique mechanisms, including metal chelation, redox disruption, and mitochondrial targeting, offer a mechanistic convergence that unites scientific intrigue with clinical relevance. This multifaceted activity is further enhanced by a well-established safety profile and decades of clinical experience in urological infections.

Yet, the path to broader clinical adoption remains hindered by regulatory inertia, PK limitations, and a lack of large-scale clinical trials. Overcoming these barriers will require a coordinated effort across disciplines and sectors. Regulatory bodies must adapt frameworks to accommodate off-patent repurposing; funders and academic institutions should prioritize clinical validation; and stewardship programs must prepare to guide evidence-based use. The story of nitroxoline illustrates both the promise and the complexity of reviving older agents in modern medicine.

Ultimately, nitroxoline is more than a candidate for repositioning. It is a case study in how mechanistic versatility, translational adaptability, and historical data can be leveraged to bridge gaps between antimicrobial resistance, oncology, and beyond. Its future will depend on not only continued scientific innovation, but also a collective willingness to reimagine the value of drugs long considered fully understood.

Author contributions

Conceptualization: **P.P.**, **H.H.**; writing – original draft: **D.B.**, **M.G.**, **H.H.**, **S.R.M.M.**, **E.P.**, **P.P.**, **K.S.**, **T.W.**; writing – review & editing: **D.B.**, **M.G.**, **H.H.**, **S.R.M.M.**, **E.P.**, **P.P.**, **K.S.**, **T.W.**; supervision: **H.H.**, **P.P.**; visualization: **H.H.**; funding acquisition: **H.H.**, **E.P.**, **K.S.**, **T.W.**

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) and LanguageTool to improve language and grammar. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of interests

All authors declare that they have no financial or personal relationships with individuals or organizations that could inappropriately influence (bias) the content of this work.

Data availability

No data was used for the research described in the article.

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